THE CONCENTRATION OF EOSIN AND THE PHOTODYNAMIC EFFECT ON TENTACLES OF A TEREBELLID WORM

JOHN H. WELSH

(From the Bermuda Biological Station for Research and the Biological Laboratories, Harvard University)

An attempt to modify the sensitivity to light of the tentacles of *Terebella magnifica* Webster by means of various dyes occasioned the use of eosin, and necessitated an investigation of its toxicity in the presence of light. The results appear to be of sufficient interest to warrant their separate consideration.

The photodynamic action of light has occupied the attention of numerous investigators since it was first reported by Raab in 1900. Reviews of the literature by von Tappeiner (1909), Clark (1922), and Blum (1932) obviate the necessity of considering the subject historically. The question of immediate interest, that of the effect of the concentration of the dye, has been considered by Fr. von Tappeiner (1908), Pereira (1925), Dognon (1928), and others, but the results in experiments on living systems are for the most part incomplete or qualitative.

Materials and Methods

The tentacular filaments of *Terebella magnifica* W. provide excellent material for such studies. Their general structure and behavior to light has been discussed in an accompanying paper (Welsh, 1934). After removal from the worm the tentacles survive for several days in sea water and when illuminated continue to move about, coiling and uncoiling, as when intact. This constant activity is of considerable importance as it stops rather suddenly at the time of death and provides a definite end point for judging the time of killing. The relative transparency of the tentacles also permits the penetration of a large part of the incident light.

Several fluorescent dyes were employed in the investigation but the results from the use of only tetrabromfluorescein or eosin Y will be considered in this account. The eosin used was a product of the National Aniline and Chemical Co., Shultz No. 587, having a total dye content of 89 per cent. This was made up in sea water to give a one per

cent stock solution by total weight. This stock solution was guarded against exposure to bright light. After a number of preliminary experiments the eosin was used in the following final dilutions; ½, 1, 5, 10, 15, 20, and 25 drops of stock solution to 25 cc. of sea water. It is unfortunate that the dilutions were not made in some other manner, as it is possible to calculate the normality only roughly; but the fact that care was used to control the drop size gave concentrations which for the purposes were sufficiently constant.

The several dilutions of eosin were placed in flat-bottomed glass dishes, 47 mm. in diameter, which gave a 15 mm. depth of solution. This thickness of the layer of eosin in sea water was a nearly constant factor, as the tentacles rested at the bottom and their movement exposed all surfaces to light of the same intensity. Sunlight was used as a

TABLE I

Killing-time in minutes for four sets of tentacles at several concentrations of eosin. (Concentration = drops of 0.89 per cent solution to 25 cc. sea water.) Temp. 32° C. ± 1°. Experiments performed in sunlight, near midday.

Concentration eosin	Time for killing				Av.
	min.	*min.*	*min.*	*min.*	*min.*
Control	—	—	—	—	—
½	60	80	50	35	56.2
1	36	35	40	30	35.2
5	28	25	22	22	24.2
10	20	18	20	20	19.5
15	23	22	25	24	23.5
20	26	25	30	27	27.0
25	30	28	32	31	30.2

source of illumination and the results reported were obtained from experiments performed on cloudless days, near midday, when the rays were essentially perpendicular. The tentacles were exposed to light immediately after being placed in the solutions.

The small containers with the eosin and tentacles were placed in a white enamelled pan and surrounded with water in order to maintain a fairly constant temperature. This averaged about 32° C. Such a temperature was somewhat higher than that normally experienced by the worms but did not appear detrimental to the controls which were run in sea water with each experiment.

RESULTS

Upon exposure to light the tentacles in all of the several dilutions of eosin exhibited greater activity than the control in sea water. They

would coil tightly and then rapidly uncoil. This continued until the epidermal cells began to plasmolyze, when the tentacles straightened, exhibited twitching movements, and soon ceased all activity. This cessation of movement was used as the endpoint and although it did not necessarily indicate the complete death and destruction of all the cells, it marked a point beyond which muscular movement did not occur. It was soon evident that this point was first reached at a concentration of ten drops of stock solution of eosin to 25 cc. of sea water. As the concentration of eosin increased or decreased in relation to this solution the killing-time increased.

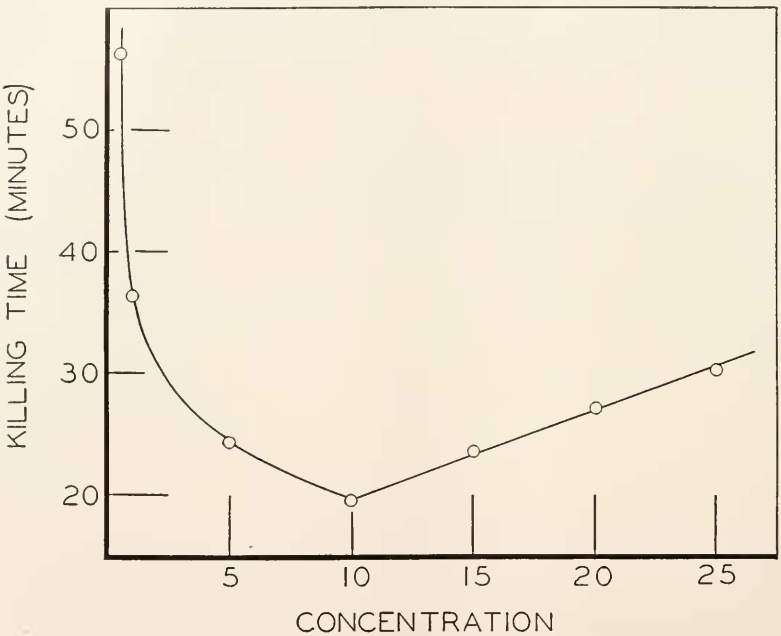

FIG. 1. The average times for killing, given in Table I, are shown plotted against the concentration of eosin. Concentration = number of drops of an 0.89 per cent solution of eosin in 25 cc. of sea water.

Table I gives the times for killing, at the several concentrations, for four separate experiments. In no instance was the control noticeably affected during the exposure although a period of illumination of several hours resulted in an earlier death of the tentacle than if the same were kept in diffuse light. It should also be mentioned that, in the diffuse light of the laboratory, tentacles would live for at least three days in the concentration of eosin which resulted in most rapid killing in direct sunlight. The averages of the times for killing are shown graphically in

Fig. 1. At the lowest concentration the average time for killing was 56 minutes. The times decreased regularly to the 10 drop concentration at which the variations were small and the average killing-time 19.5 minutes. A smooth curve may be drawn through the points obtained and the curve would approach more closely the abscissa were it not for a secondary process entering in which causes an increase in the killing-time. The secondary process is unquestionably due to a shielding effect resulting from an absorption of light by the overlying layer of eosin. It probably follows Beer's Law for the absorption of light. Exceptions to this law are known, but in general the absorption of light by a solution depends on the molecular concentration if the thickness of the absorbing layer is kept constant. This screening effect, which begins to reduce the photodynamic action of the eosin, results in an increase in killing-time and the averages now bear a linear relationship to the concentration over the range employed. If the thickness of the layer of eosin were varied, it is probable that the relationship between concentration and killing-time at the higher concentrations would vary considerably. However, another variable, that of the diffusion of oxygen, would enter in to complicate the matter, as the importance of oxygen in photodynamic processes has been repeatedly demonstrated.

The data of Fr. von Tappeiner (1908) obtained from a study of the effect of concentration of eosin on the hemolysis of red blood cells are in essential agreement with the above. A minimum time for hemolysis of 17 minutes was found at a concentration of 1/2000 N eosin. At concentrations above and below this the times increased. At a concentration of 1/40,000 N the time had increased to 44 minutes and at the highest concentration of 1/200 N the time for hemolysis was 34 minutes. The observations of Pereira (1925) on the combined toxic action of eosin and light on *Arbacia* eggs, sperm, and larvae were qualitative only, but indicated a definite effect of concentration of the dye. Dognon (1928) studied the effect of the concentration of several fluorescent dyes on the killing of paramecia and obtained a definite relationship between concentration and killing-time although he employed only concentrations below that which produced killing in a minimum time.

Results by Gros (1901) on the rate of bleaching of fluorescein in light indicate that at a given concentration the rate of bleaching is at a maximum and that increase or decrease in concentration produces a decrease in rate of bleaching. Blum (1932) mentions other such experiments on non-living systems, all of which yielded essentially similar results.

It would be of interest to employ screens of eosin varying in con-

centration, while keeping tentacles in a given concentration, in order to separate the absorption effect from the photodynamic action. Results obtained in this manner should yield to more precise analysis and permit the formulation of significant mathematical equations to explain the combined effect.

It is evident from the results obtained that the concentration of the fluorescent material is a limiting factor in determining the rate of a photodynamic process. When all other factors are constant the photodynamic effect increases with the concentration of the dye up to a certain point, beyond which there is a decrease due to the absorption of light by the layer of dye. Over the range of concentrations employed in the present study the times for killing, above the minimum, are apparently directly proportional to the concentration of the sensitizing material. Over a wider range of concentrations this relationship probably does not hold, as appears to be indicated by other similar investigations.

Summary

The tentacles of *Terebella magnifica*, W. are satisfactory for the study of certain photodynamic phenomena. The toxic effect of eosin in the presence of light depends upon the concentration of the dye. As the concentration increases, the killing-time decreases to a minimum and then increases linearly over a certain range. This secondary increase is probably due to absorption of light by the overlying layer of eosin.

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